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ANALYTICAL REFERENCE SERVICE TRAINING PROGRAM. MILK DDT-RESIDUE, NUMBER 1, JUNE 1962

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

June 1962

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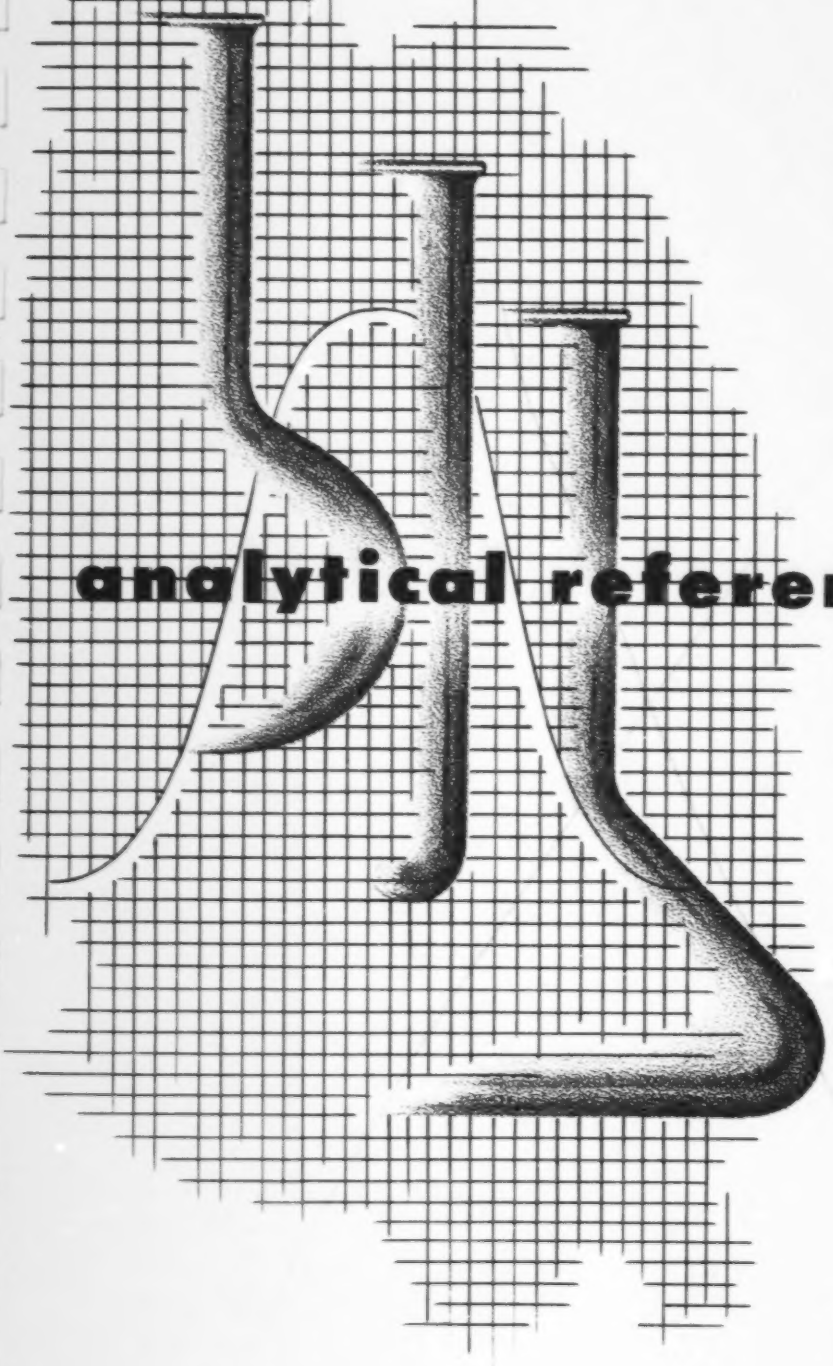

MILK
DDT-RESIDUE NO. 1
JUNE, 1962

analytical reference service

TRAINING PROGRAM

Robert A. Taft

SANITARY ENGINEERING CENTER

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CINCINNATI, OHIO

PB 227 826

Report of

ANALYTICAL REFERENCE SERVICE

MILK DDT-RESIDUE NO. 1

Conducted by the

Analytical Reference Service

TRAINING PROGRAM

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

Public Health Service
Bureau of State Services
Division of Environmental Engineering and Food Protection

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1962

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ACKNOWLEDGEMENT

The Analytical Reference Service Staff gratefully acknowledges the assistance of L. A. Richardson, Ph. D., Biochemist, Milk and Food Training Activities, Training Program.

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FOREWORD

The Analytical Reference Service is conducted by the Public Health Service at the Robert A. Taft Sanitary Engineering Center to provide a means of evaluating laboratory methods in the field of environmental sciences and engineering. Membership includes federal, state, and municipal agencies, industries, universities, consulting firms, and foreign agencies. Cooperative studies by member organizations, through analysis of identical samples of environmental media and critical review of methodology, provide the mechanism for:

1. Statistical evaluation of procedures, including precision and accuracy.

2. Comparison of analytical procedures and results between laboratories having similar responsibilities.

3. Exchange of information regarding weaknesses of currently approved methods.

4. Further development of methods or entirely new approaches.

5. Broad-scale evaluation of new analytical methods.

While largely directed toward the chemical examination of water, the studies have been broadened gradually to include radioactivity, air pollution, and milk and food contamination. Samples are designed and prepared to contain measured amounts of selected constituents. Decisions as to qualitative makeup are made by the staff of ARS, the membership, and consultants. Notice of a study is forwarded to each member with information regarding the qualitative makeup of the sample and special details regarding its handling.

A portion of the sample is shipped to interested members with accompanying data forms which provide space for numerical analytical values and a narrative critique of the procedures used. The laboratories are urged to comment on the methods, difficulties, modifications, sources of error, and other factors. Results are usually requested within sixty days from receipt of the sample, though in this study, it was necessary to establish a two-week deadline to assure stability of the milk sample.

The results and comments of each study are compiled, as in the accompanying report in which 14 agencies contributed data. Experience indicates that a repeat study produces a sizeable increase in the number of participants. For example, in the 1956 water-minerals study, 19 agencies participated; in the 1958 study, 31 member agencies participated; 59 agencies cooperated in the 1961 study.

A sample for the determination of strontium 90-yttrium 90 in water has just recently been shipped. A pilot study is now being conducted in the ARS Lab. to produce a stable SO_2 in Air sample for distribution to interested members. A phenol in water study is also being planned.

Selection of these studies was guided by a poll of member interests. It will continue to serve as a basis for future studies.

The steadily increasing membership, now 141 agencies, strongly indicates the broad interest in and need for this type of service.

H. P. Kramer

H. P. Kramer
Chief, Training Program

STUDIES ON WHICH REPORTS HAVE BEEN COMPLETED

Water-Minerals	Calcium, magnesium, hardness, sulfate, chloride, alkalinity, nitrite, nitrate, sodium, and potassium. Studies completed in 1956, 1958, and 1961.
Water-Metals	Lead, copper, cadmium, aluminum, chromium, iron, manganese, and zinc. First study completed in 1957. Report on second study to be distributed in the near future.
Water-Fluoride	Fluoride in the presence and absence of interferences, with and without distillation using a specified procedure. Studies completed in 1958 and 1961.
Water-Radiological	Gross beta and gamma activity. Studies completed in 1959 and 1961.
Water-Surfactant	Surfactant in various waters. Study completed in 1959.
Water Oxygen-Demand	Biochemical oxygen demand and chemical oxygen demand. Study completed in 1960.
Water Trace-Elements	Study completed in 1962. Constituents were arsenic, boron, selenium, and beryllium.
Air-Inorganics	Chloride, sulfate, fluoride, and nitrate in aqueous solution and on glass fiber hi-vol filter mats. Study completed in 1958.
Air-Lead	Filter paper tape impregnated with lead. Study completed in 1961.

PARTICIPANTS IN THIS STUDY

ARS MEMBERS

California State Department of Public Health, Los Angeles
Colorado State Department of Public Health
Maryland State Department of Health
Metropolitan Utilities District, Omaha, Nebraska
New Jersey State Department of Health
Case Institute of Technology, Dept. of Civil and Sanitary Engineering
Texas State Department of Health

GUEST PARTICIPANTS

Communicable Disease Center, Atlanta, Georgia
Military Subsistence Testing Laboratory, U. S. Army, Chicago
New York City Department of Health
M & R Dietetic Laboratories, Inc., Columbus, Ohio
Pennsylvania Department of Agriculture, Harrisburg
Washington State Food & Drug Laboratory, Seattle
Wisconsin Department of Agriculture, Madison

MILK DDT-RESIDUE NUMBER 1

PURPOSE OF THE STUDY

This study, the first for quantitative determination of DDT in milk, was designed to permit evaluation of the precision and accuracy obtainable by using the AOAC Official Method for the Colorimetric Determination of DDT Residues, and to obtain data which would be helpful in planning future studies for the evaluation of other methods. At the request of the Analytical Reference Service, all participants were volunteer agencies having responsibilities in the field of milk and food contamination. Provision was made for the participants to critique the procedure.

PREPARATION OF THE SAMPLE

The master sample consisted of 22 liters of pasteurized homogenized milk purchased from a grocery store. No DDT was detectable in the preliminary examination.

The milk sample was mixed in a 40-liter pyrex glass container for 30 minutes at room temperature, using a magnetic stirrer. A 500-ml portion was removed and held for analysis (Control No. 1). To the remainder, 32.5 ml of 40% formaldehyde solution was added, providing 0.15% formalin as the preservative.

The sample was mixed for an additional 30 minutes and a second 500-ml aliquot was removed (Control No. 2). 13.61 mg of Technical DDT (77.2% para para' DDT), dissolved in 100 ml of ethanol, was added to the master sample to provide a para para' DDT concentration of 0.50 mg/l. Following the "spiking", the master sample was mixed for an additional 3 hours. Aliquots were then withdrawn and shipped in polyethylene bottles to participating agencies.

A third control sample (Control No. 3) was prepared by using a portion of Control No. 1 and adding ethanol to the same concentration as that of the master sample. A final control sample (Control No. 4) was prepared by adding ethanol to a portion of Control No. 2 to give the same ethanol concentration as in the master sample.

Ethanol was used to dissolve the DDT because it is believed that a solvent miscible with the aqueous phase of milk effects a more homogeneous distribution of the insecticide than does an aqueous-immiscible organic solvent. It is also believed that ethanol is least likely to inter-

fere with the analysis since it is removed very early in the procedure.

On analysis, Controls 1 through 4 yielded negative results, verifying the absence of detectable DDT in the original milk and indicating that the presence of formaldehyde and ethanol do not interfere with the analytical procedure. It was noted, however, that the presence of formaldehyde does somewhat increase the difficulty of the initial extraction.

Analysis of the completed sample by the Milk and Food Training Activities staff after one-week storage at room temperature, yielded the following results:

	DDT Concentration, mg/liter
	0.51
	0.48
	0.46
	0.43
Avg.	0.47

These results, together with preliminary work[3], demonstrate that fat-soluble insecticides can be homogeneously incorporated into milk by the procedure outlined.

A supply of a DDT Reference Standard was shipped to all participants to insure identical standards.

TREATMENT OF THE DATA

Participants reported results in triplicate to the nearest one hundredth of a milligram per liter. Table No. 1 lists data reported by all agencies, the arithmetic mean (average) value for each participant, percent deviation within each laboratory (precision), and the percent deviation from the amount added (accuracy).

Precision within each laboratory was obtained by calculating the average percent deviation. This, in turn, was obtained by averaging the deviations of the replicate values from the mean, dividing by the mean, and expressing the result as percent deviation. The 95% confidence limit is based on the standard deviation and represents the range of deviation from the calculated mean within which the true mean of an infinite number of determinations will fall 95% of the time.

Accuracy is represented as percent deviation from the amount of DDT added to the sample. This percent deviation was computed by averaging the deviations of the individual results from the amount added (0.50 mg/l), dividing this average by the amount added, and expressing as percent deviation.

A Summary of computations from the data in Table 1 is listed below:

Amount of DDT added	0.50 mg/l
Mean	0.39 mg/l
Median	0.40 mg/l
Standard deviation	±0.150 mg/l
Range	0.10-0.70 mg/l
95% confidence limit	±0.49 mg/l
No. of participants	14

DISCUSSION OF RESULTS

Seven of the 14 participating agencies routinely analyze for DDT residues in milk. Of this group, 5 normally use the paper chromatographic procedure for milk[2], one uses the Official AOAC Colorimetric Procedure[1], and one a modification of the latter method, sometimes referred to as the Schechter-Haller procedure[4]. All participants em-

ployed the specified AOAC method in this study, although most laboratories modified the method somewhat.

The variation in results, shown by the accompanying data, was anticipated, since this procedure is new to many of the participating laboratories, and the method itself is quite long, involved and exacting.

Reasons for the high results reported by two of the laboratories are not immediately evident; however, such results may be due to incomplete "cleanup", specifically to incomplete removal of ring compounds prior to nitration. Data necessary for a more complete explanation of the high results are not available.

Low results, on the other hand, were probably due to losses of residue during analysis. Since the AOAC is an involved procedure, there are many opportunities to lose a portion of the residue, particularly during the "cleanup". Many of these laboratories indicated considerable emulsion difficulty in the initial extraction, with the suggestion that complete extraction of the pesticide residue was not achieved. Difficulties were reported with the preparation and functioning of the acid-celite adsorption column, which could be responsible for incomplete elution and low results. One laboratory noted that water and an accompanying yellow color were being eluted from the adsorption column indicating improper preparation of the column. Other participants also reported the elution of yellow color.

Preparation of the sodium methylate color-development reagent created some difficulty. While none of the laboratories were troubled with off colors in this solution, a turbid condition was encountered in some instances, which might have resulted from too rapid preparation, use of old or impure reagents, or the presence of excessive amounts of carbonate. The use of such a reagent could conceivably influence the results even though it was used in both standards and samples.

Other conditions, which could have influenced the results in either direction, include the more common laboratory errors of measurement, calculation, and instrumental evaluation, including errors in preparation of the standard curve.

It was interesting to note the number of laboratories reporting about half the theoretical DDT concentration. Since certain laboratories used centrifuge cups, in the extraction step, which were just half the recommended size, some question exists as to whether or not the amount of sample analyzed was also half the recommended amount. Such a procedure could result in a final calculation of about half the DDT concentration.

Consensus of the participating laboratories is that the AOAC method

is long, laborious, tedious, and exacting, and is of value only as a means of determining the concentration of DDT in samples known to be contaminated. It should be noted that this method was developed specifically for this purpose; it was not intended to be used as a "screening" procedure.

Apparently the principal weakness in the procedure, aside from the time required, is the initial extraction from milk. All laboratories, including the SEC laboratory, experienced problems of emulsions. Certain participants applied modifications which may have somewhat improved the DDT extraction, while one laboratory used a deviation in the extraction procedure undoubtedly responsible for very poor recovery. It is doubtful however, that the admitted weakness of the extraction step of this procedure is responsible for the large number of low results.

The data indicate that considerable training and experience are necessary in the area of pesticide residue analysis, particularly in the application of the method used in this study.

SUMMARY OF DATA
BAR CHARTS

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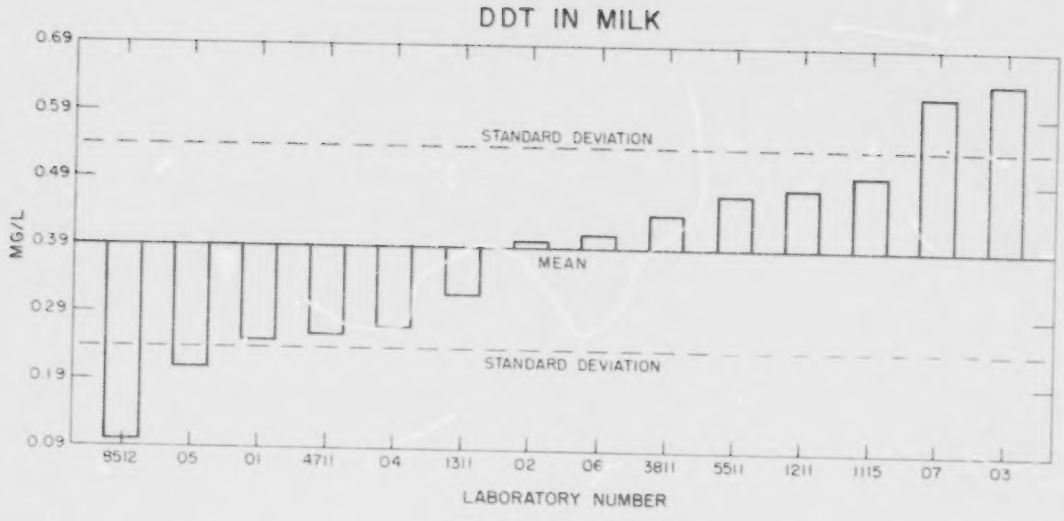
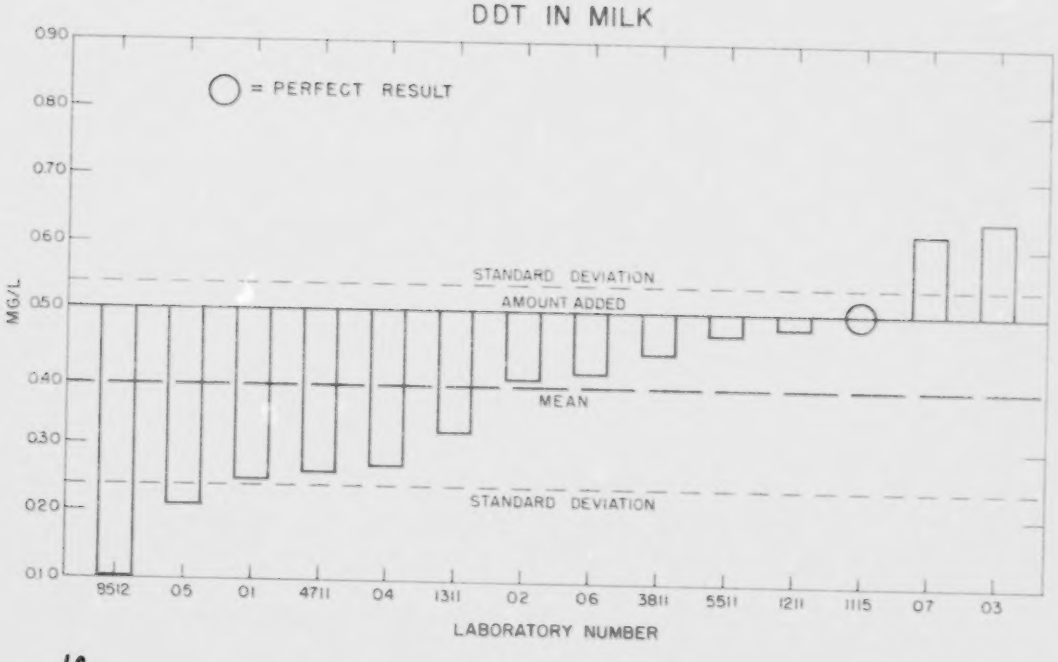
Table 1. Summary of Data (0.50 mg/l added)

Lab. No.	Results	Mean	Precision (% dev. within labs.)	Accuracy (% dev. from amt. added)
1115	0.50 0.45 0.55	0.50	6.67	6.67
1211	0.49 0.47 0.47	0.48	2.08	4.67
1311	0.31 0.33 0.36 0.29	0.32	7.03	35.50
3811	0.44 0.44 0.44	0.44	0	12.00
4711	0.28 0.26 0.25	0.26	3.85	47.33
5511	0.47	0.47	0	6.00
8512	0.10	0.10	0	80.00

Table 1 (Continued)

Lab. No.	Results	Mean	Precision (% dev. within labs.)	Accuracy (% dev. from amt. added)
01	0.24 0.23 0.27	0.25	6.66	50.67
02	0.30 0.44 0.46	0.40	16.66	20.00
03	0.70 0.62 0.59	0.64	6.77	27.33
04	0.26 0.25 0.29	0.27	6.17	46.67
05	0.21 0.21 0.21	0.21	0	58.00
06	0.40 0.40 0.44	0.41	4.06	17.33
07	0.60 0.63 0.63	0.62	2.15	24.00


DDT IN MILK
0.69
0.59
0.49
0.39
0.29
0.19
0.09
MG/L
STANDARD DEVIATION
MEAN
STANDARD DEVIATION
8512
05
01
4711
04
1311
02
06
3811
5511
1211
1115
07
03
LABORATORY NUMBER


DDT IN MILK
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
MG/L
= PERFECT RESULT
STANDARD DEVIATION
AMOUNT ADDED
MEAN
STANDARD DEVIATION
8512
05
01
4711
04
1311
02
06
3811
5511
1211
1115
07
03
LABORATORY NUMBER

COMMENTS OF PARTICIPANTS

LABORATORIES

No. 1115

Sodium Methylate Solution - We used $NaOCH_3$ and could not obtain an amber color even on a hot plate. Other forms may result in an amber color, however. Specifying form may save much time. The ice bath was not necessary.

Centrifuge - A centrifuge with a capacity for 8-50 ml tubes was used, obviously involving many more quantitative transfers and increasing the possibility of error. A siphon as shown, attached to the method we received, was not used. Instead, the ether layer was extracted with a pipette.

Where "redistilled petroleum ether" was called for, we used Fisher's petroleum ether, Cat. No. E-139. The AOAC method is extremely long and tedious. Two successive runnings of the standard curve both resulted in the formation of a white precipitate in the 40 and 50 µg tubes which were insoluble in benzene, step #24. The color seemed to be less well developed in these tubes. Determination of DDT in Milk not routinely performed.

No. 1211

The AOAC method is time-consuming and exacting. The deviation from method was the use of water rather than milk as a blank, beginning with the extraction of milk. The AOAC Organic Chlorine method is preferred. This determination not routinely performed.

No. 1311

Due to the difficulties encountered in the method accompanying the sample, several modifications were made. Difficulties encountered were: (1) formation of a gelatinous emulsion not broken by centrifugation (2) in preparation of acid-celite column, slurry could not be sufficiently compressed (3) the sodium methylate solution when prepared was quite turbid, though colorless. Modifications to correct the above difficulties were employed. This laboratory routinely analzes for DDT in milk, employing the Mills' Paper Chromatography procedure.

No. 3811

The AOAC method too long, involved, and costly for routine analysis. This analysis not routinely performed but prefer Mitchell's (Mills') paper Chromatography method.

No. 4711

Method found to be time-consuming due to our inexperience with it, to lack of large centrifuge head and cups, and lack of air supply in hood. The column separation of the fat with Acid-celite appears to be much better than the acetonitrile-florisil procedure. Deviations from AOAC method employed were: in the fat extraction, colloidal gels difficult to break formed, we transferred sample to one-liter separatory funnels. Heat was applied by allowing hot water to run over the funnels and sodium sulfate solution was added. After noting that the sample smelled sour, addition of anhydrous potassium carbonate made separation possible. This analysis routinely performed by a paper chromatographic procedure, Paul Milis, Journal Association of Agricultural Chemists, November 1959.

No. 5511

There is strong doubt that the celite column gives sufficient clean-up on the fat from 100 gms of milk. The contamination which rendered an off-color in replicates 2 and 3 apparently occurred during the clean-up part of the method. No difficulty was encountered in preparation of standards. The Mills method for extraction of the milk fat was used in this determination. Analysis for DDT in milk is routinely done and paper chromatography is used.

No. 8512

The alcohol-petroleum ether extraction of the milk difficult. An emulsion formed which was very hard to break. Remainder of method, including calibration curve, worked out nicely. We deviated from the AOAC method by using a stirring and decantation process which probably did not provide complete extraction. This was necessary due to the lack of the correct centrifuge head. This analysis is not routinely performed.

No. 01

Air pockets were encountered in the preparation of the sulfuric acid-celite column which could not be removed by packing down with the tamping rod. Columns then were prepared dry as de-

scribed in AOAC Methods of Analysis, 9th Edition, p. 345, paragraph 24:111 (1960) and then wetted with C Cl_4. Twice the amount of redistilled petroleum ether was used in the initial extraction of DDT and fat from the milk sample. This laboratory does not routinely test for DDT.

No. 02

We do not analyze for DDT in milk routinely.

No. 03

Severe emulsions were encountered with this method of fat extraction. The sample was received late which may account for difficulty. The clean-up procedure with the acid-celite column was thorough. It was necessary to add a small amount of potassium oxalate and 95% ethyl alcohol to break the emulsion. We routinely use the paper chromatographic method of Mills for screening. A chromatographic run was made on this sample, yielding a value of 0.1 mg/l. Emulsions were encountered.

No. 04

In the extraction step, we found the emulsions very hard to break. More than 3 drops of HCl were needed. We routinely use the Schechter-Haller method.

No. 05

If 250 ml centrifuge bottles are used, all reagents except petroleum ether should be halved. If 25 ml are used, will give poor separation and recovery. The C Cl_4 eluate from the Davidow column should be dried with anhydrous sodium sulfate before evaporation.

The petroleum ether solution of nitrated products should be washed at least 6 times with 10% KOH to remove yellow color. Deviations from the AOAC method were: 100 gm sample divided between 2-250 ml centrifuge bottles and 50 ml petroleum ether was used to extract each for all extractions. Chromatographic column was 32 mm × 225 mm I. D. The DDT analysis is routinely performed by Mills' procedure, gas chromatography, and by AOAC colorimetric after "clean-up by Mills procedure.

Although our results were in close agreement, we feel they are in error because of the amount of yellow color carried over to the final nitrated product. The sample was received partially frozen.

No. 06

Final heating at 100°C for one hour seemed to eliminate possibility of off-colors. We like the method. Column preparation as per McRoberts, Bureau By-Lines, No. 2, October, 1961. We used glass stoppered test tubes in place of 50 ml erlenmeyer flasks. We think this is more convenient. We routinely use the AOAC method for DDT confirmatories. We doubt our results due to a fire which caused the loss of reagents and the spectrophotometer normally used.

No. 07

More HCl and alcohol had to be added to break the emulsion before centrifuging. This is not a routine analysis in this laboratory.

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1. Association of Official Agricultural Chemists, Methods of Analysis, 9th Ed., pages 341-345, 1960.

2. Mills, Paul A. Detection of Semiquantitative Estimation of Chlorinated Organic Pesticide Residues in Food by Paper Chromatography, Journal Assn. Official Agricultural Chemists. 42:734-40, 1959.

3. Richardson, L. A. and Jackson, M. D. Unpublished data.

4. U.S. Public Health Service, Methods for the Chemical Analysis of DDT and DDE in Samples of Fat, Chemical Memorandum No. 1, TB9-1-53, 1st Revision, U.S.P.H.S., TDL, P. O. Box 769, Savannah, Georgia. (Schecter-Haller)

ANALYTICAL REFERENCE SERVICE MEMBERS

STATE AGENCIES

Alabama Water Improvement Commission
Arizona State Department of Health
Arkansas State Board of Health
California State Department of Public Health (Berkeley)
California State Department of Public Health (Los Angeles)
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Pearl Harbor Naval Shipyard
U.S. Army Environmental Hygiene Agency, Maryland
U.S. Department of the Interior, Bureau of Reclamation, Denver, Colorado
U.S. Department of the Interior, Geological Survey, Sacramento, California
U.S. Department of the Interior, Geological Survey, Denver, Colorado
U.S. Department of the Interior, Geological Survey, Columbus, Ohio
U.S. Department of the Interior, Geological Survey, Philadelphia, Pennsylvania
2794th U.S. Air Force Dispensary - Class B

UNIVERSITIES

Case Institute of Technology, Department of Civil and Sanitary Engineering
Columbia University
University of Florida, Department of Chemistry
Georgia Institute of Technology, Department of Applied Biology
Johns Hopkins University, School of Hygiene and Public Health
University of Kansas, School of Engineering and Architecture
University of Pittsburgh, Graduate School of Public Health

Purdue University, Department of Chemistry
Rutgers - The State University, Department of Sanitation
Washington State University, Division of Industrial Research

INDUSTRY

American Cyanamid Company, Bound Brook, New Jersey
Anaconda Company, Grants, New Mexico
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Ottawa, Ontario, Canada, Occupational Health Division, Department of National Health and Welfare

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